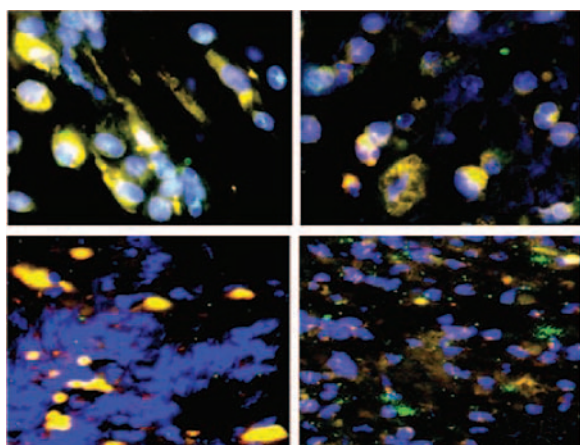


Diabetic impairments in NO-mediated endothelial progenitor cell mobilization and homing are reversed by hyperoxia and SDF-1 α

Poor wound healing in diabetic patients is characterized by impaired angiogenesis and vasculogenesis. The bone marrow-derived endothelial progenitor cell (EPC) is a key cell involved in vasculogenesis and homes to peripheral tissue in response to ischemia. Previous studies have begun to elucidate the mechanisms responsible for the mobilization of EPCs into the circulation and their recruitment into areas of peripheral tissue ischemia. However, it remains unknown why the main physiological stimulus for EPC mobilization and recruitment (that is, ischemia) fails to induce therapeutic EPC-mediated neovascularization and healing in wounds of diabetic hosts.



Gallagher et al./J Clin Invest

Decreased SDF-1 α expression in peripheral wounds of diabetic mice.

It is known that the EPC mobilization cascade starts with peripheral hypoxia-induced tissue release of vascular endothelial growth factor-A and subsequent activation of bone marrow stromal nitric oxide synthase (NOS), resulting in increased bone marrow nitric oxide (NO) levels. This results in the mobilization of EPCs from the bone marrow to circulation, allowing for their participation in tissue-level vasculogenesis. At the tissue level, EPC recruitment depends on ischemia-induced upregulation of stromal cell-derived factor-1 α (SDF-1 α).

In a recent study, Gallagher *et al.* examined the effect of diabetes in these processes. They found that diabetic mice had impaired phosphorylation of endothelial NOS in the bone marrow, decreased circulating EPCs, and diminished SDF-1 α expression in cutaneous wounds (Figure). Interestingly, hyperoxia increased bone marrow NO and circulating EPCs, effects that were inhibited by the NOS inhibitor N-nitro-L-arginine-methyl ester. In addition, administration of SDF-1 α into wounds reversed the EPC homing impairment and, with

hyperoxia, synergistically enhanced EPC mobilization, homing, and wound healing. Thus, hyperoxia reversed the diabetic defect in EPC mobilization, and SDF-1 α reversed the diabetic defect in EPC homing. (*J Clin Invest* 2007; 117: 1249–1259)

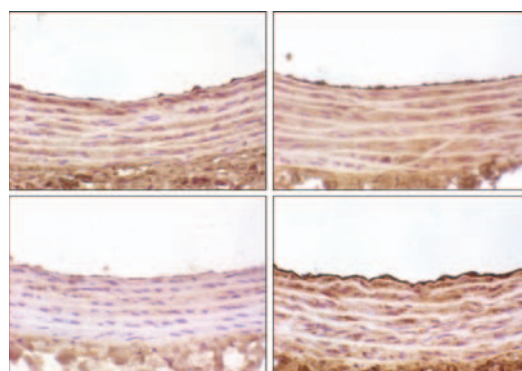
Juan Oliver

Angiotensin II upregulates soluble epoxide hydrolase

Arachidonic acid can be converted to eicosanoids by three major enzymatic pathways: cyclooxygenase, lipoxygenase, and CYP 450 epoxygenase. Four epoxyeicosatrienoic acids (EETs) are the major metabolites generated by CYP 450 epoxygenase. EETs in endothelial cells exert autocrine effects but can also act as paracrine mediators on neighboring cells such as vascular smooth muscle cells. EETs exert many other effects; they can function as endothelium-derived hyperpolarizing factors and by increasing intracellular Ca²⁺ concentration in vascular smooth muscle and activate large-conductance Ca²⁺-activated K⁺ channels, causing hyperlocalization and lowering blood pressure.

Under physiological conditions, EETs can be enzymatically hydrolyzed to dihydroxyeicosatrienoic acids by epoxide hydrolases (EHs). Two major EHs exist in mammalian cells: soluble EH (sEH), which primarily presents in the cytosol and peroxisomes, and microsomal EH, which binds to the intracellular membranes. Soluble EH is highly expressed in the liver, kidney, intestine, and vasculature. In past studies, administration of selective inhibitors of sEH to angiotensin II-infused hypertensive rats greatly increased the level of EETs and lowered systolic blood pressure. In addition, a selective sEH inhibitor also reversed the hypertension in the spontaneously hypertensive rat. Thus, augmentation of EET levels by sEH seems to control blood pressure *in vivo*.

To examine the interaction between angiotensin II and EETs, Ai *et al.* treated endothelial cells with angiotensin II and found increased sEH expression (Figure). Transient transfection assays showed that the activity of the human sEH promoter increased



Ai et al./Copyright 2007 National Academy of Sciences, U.S.A.

The level of sEH protein is elevated in the aortic intima of spontaneously hypertensive rats.

in endothelial cells in response to the hormone. Analysis of the promoter region of the sEH gene demonstrated that angiotensin II activates the sEH promoter through an activator protein-1 (AP-1)-binding motif. They also found elevated levels of sEH in the aortic intima of both spontaneously hypertensive rats and angiotensin II-infused normal rats. Further, losartan abolished the sEH induction. Thus, AP-1 activation is involved in the transcriptional upregulation of sEH by angiotensin II in endothelial cells, which may contribute to angiotensin II-mediated hypertension. (*Proc Natl Acad Sci USA* 2007; **104**: 9018–9023)

Juan Oliver

Bradykinin B1 and B2 receptors both have protective roles in renal ischemia/reperfusion injury

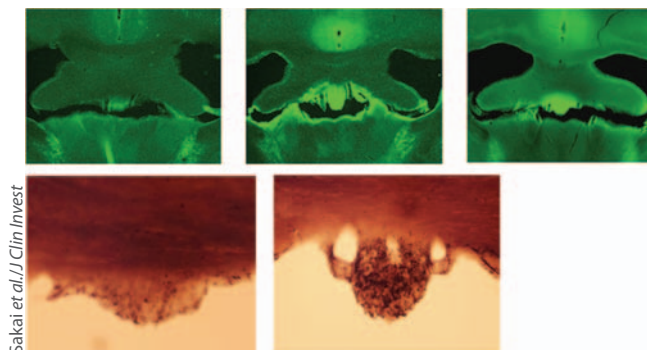
Angiotensin I-converting enzyme inhibitors (ACEIs) not only slow the progression of chronic renal diseases but are also protective after acute injury caused by ischemia/reperfusion in several organs, including the kidneys. Several observations indicate that the beneficial effects of ACEIs in these acute models of disease are mainly due to activation of the bradykinin–nitric oxide cascade as a result of inhibition of the inactivation of bradykinin. Thus, ACEIs have a greater effect than angiotensin type I receptor antagonists, and both bradykinin B2 receptor (B2R) antagonists and NOS blockers markedly attenuate the acute protective effects of ACEIs.

In mammals, at least two receptors have been identified: the bradykinin B1 receptor (B1R) and the B2R. The stimulation of both receptors activates endothelial NOS in the vascular endothelium.

In contrast to the consensus that ACEIs have beneficial effects on ischemia/reperfusion injury, previous studies have produced conflicting results concerning the effect of stimulating the two bradykinin receptors. To clarify these issues, Kakoki *et al.* generated mice lacking both the B1R and the B2R for comparison with wild-type mice and with mice lacking the B2R only. They found that mortality rates, renal histological and functional changes, 8-hydroxy-2'-deoxyguanosine levels in total DNA, mitochondrial DNA deletions, and the number of TUNEL-positive cells in the kidneys increased progressively after 30 minutes of bilateral renal artery occlusion and 24 hours of reperfusion. The increases occurred in the following order (from lowest to highest): wild type, B2R-null, and B1RB2R-null mice. Increases in mRNA levels of transforming growth factor- β 1, connective tissue growth factor, and endothelin-1 after ischemia/reperfusion injury were also exaggerated in the same order. Thus, both the B1 and the B2 bradykinin receptors play an important role in reducing DNA damage, apoptosis, morphological and functional kidney changes, and mortality during renal ischemia/reperfusion injury. (*Proc Natl Acad Sci USA* 2007; **104**: 7576–7581)

Juan Oliver

Local angiotensin II generation in the subfornical organ increases drinking



Localization of angiotensin peptide in the brain of SRA and SRA^{flox} mice.

The renin–angiotensin system is known to play a critical role in body fluid regulation through its actions within the kidney and central nervous system. Direct intracranial or intracerebroventricular injection of angiotensin II causes increases in blood pressure and dipsogenic behavior. Furthermore, centrally administered angiotensin II receptor antagonists are known to eliminate the cardiovascular effects caused by exogenous hormone. Although these experiments have provided evidence that the central nervous system is a target for the pressor and dipsogenic actions of exogenous angiotensin II, it remains unclear how endogenous hormone is formed within the brain, where it is located, and how regional angiotensin II production correlates with specific cardiovascular responses.

In the brain, the subfornical organ (SFO) has long been known to be an important cardiovascular control region regulating fluid homeostasis in response to angiotensin II. However, the SFO lies outside the blood–brain barrier and thus has access to circulating and intraventricular angiotensin. However, angiotensinogen (AGT) is abundantly expressed in the SFO. In a recent study, Sakai *et al.* examined the effects on fluid homeostasis of regional production of angiotensin II derived from local renin (REN) and angiotensinogen in the SFO (Figure). They used genetic techniques that allowed for spatiotemporal control of gene expression and ablation. They found that targeted expression of *hREN* and *hAGT* resulted in a marked increase in water and salt intake, which significantly decreased in response to the blockade of angiotensin receptors in the brain. The mice exhibited elevated angiotensin II, specifically in the SFO. SFO-specific ablation of *hAGT* synthesis by Cre recombinase resulted in a significant decrease in *hAGT* and angiotensin II immunostaining in the SFO and correlated with a significant decrease in water intake. These results provide solid genetic evidence that local *de novo* synthesis of angiotensin II in the SFO plays an integral role in regulating fluid homeostasis and thirst. (*J Clin Invest* 2007; **117**: 1088–1095)

Qais Al-Awqati